



Biology and Integrated Control of Tomato Wilt Caused by *Fusarium oxysporum lycopersici*: A Comprehensive Review under the Light of Recent Advancements

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Abstract

Tomato (*Lycopersicon esculentum* L.) is one of the most popular and important commercial vegetable crops grown throughout the world. Tomato is a rich source of various micronutrients and antioxidants. One of the major causes of poor quality and fruit loss during storage and transport are diseases caused by phyto-pathogenic fungi. Fusarium wilt of tomato caused by *Fusarium oxysporum lycopersici* becomes one of a limiting factor in the production of tomato and accounts for yield losses in Jordan and the whole world. It is considered as one of the most prevalent and damaging diseases wherever tomatoes are grown because the pathogen persists in infested soils. Pathogenic fungi of the genus *Fusarium*, cause root and basal stem deterioration and result in the wilting of vegetable plants. Browning of the vascular tissue is a strong evidence of fusarium wilt. Because of hazards of pesticides in general, and fungicides in specific, on public health and environmental balance, new direction of pest control management was introduced. Biological management of Fusarium wilt of tomato using bio-fortified composts associated with selected biological control agents (Fungal and bacterial species) is studied. Recently, different reports stated that some plant extracts and plant essential oils have been reported to be effective antimicrobial agents against food and stored grain fungi, foliar pathogens, and soil-borne fungal phyto-pathogens. This review is aimed at investigating the occurrence of Fusarium wilt of Tomato in Jordan and summarizing the best ways of management and control, especially the biological means.

Keyword

Fusarium oxysporum, Fungicide, Tomato, Fusarium wilt, Plant extract

Introduction

Tomato (*Lycopersicon esculentum* L.), which belongs to family *Solanaceae* and genus *Solanum* [1-5] is native to South America and was first discovered in Mexico [6]. The cultivated form was first taken to Europe by Spanish in the 16th century and from there introduced into southern and eastern Asia, Africa and the Middle East and distributed throughout the world [7-10]. According to Al-Shadiadeh, et al. [10], world production exceeds 133 million metric tons which covered around 4.7 million hectares from the whole production in 2007.

Tomato is considered as one of the highly valuable nutritive and widely grown vegetables in 144 countries of the world. The most tomato producing countries of the world in metric ton production are China, United States, Turkey, India and Egypt. The total area under cultivation of tomato is 45,82,438 thousand ha. Whereas, the production 15051381 thousand tones and productivity of 32.8 tones/ha has been recorded in the world [11]. China is considered as the largest area of production in the world which occupying 871,235,000 hectares with a production of 41,879,684,000 tonnes [4].

But the highest productivity is come out from USA which is 81 ton/ha. In India, the total production of tomato is 16826 thousand tons which comes from 865 thousand hectares of land [12].

In Egypt, tomato is the most important vegetable crop. About 186.000 ha is cultivated and the average production reaches up to seven million tons, which are consumed either fresh or processed [13]. Economically tomato is the second most important vegetable produced worldwide at around 115.95 million tons per year as it is a short duration crop and gives a high yield, it is economically attractive and the area

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Table 1: Most Common Pests and their Causal Agents which Attacks Tomato in Jordan [154].

Fungi	
Fusarium wilt	<i>Fusarium oxysporum</i>
Black mold	<i>Alternaria alternata</i>
Early blight	<i>Alternaria solani</i>
Bacteria	
Bacterial canker	<i>Clavibacter michiganensis</i>
Bacterial speck	<i>Pseudomonas syringae</i>
Oomycetes	
Buckeye rot (Phytophthora root rot)	<i>Phytophthora spp.</i>
Late blight	<i>Phytophthora infestans</i>
Viruses	
Tomato mosaic virus	<i>Tomato mosaic virus (ToMV)</i>
Tomato spotted wilt	<i>Tomato spotted wilt virus (TSWV)</i>
Tomato Yellow Leaf Curl disease	<i>Tomato Yellow Leaf Curl Virus (TYLCV)</i>
Insects	
Beet armyworm	<i>Spodoptera exigua</i>
Tomato fruit worm (Corn earworm)	<i>Helicoverpa zea</i>
Nematodes	
Root knot nematode	<i>Meloidogyne spp.</i>
Mites	
Spider mites (Two-spotted spider mite)	<i>Tetranychus urticae</i>

under cultivation is increasing. Its position in the whole world is after potato and sweet potato both in area and production [14,15]. In Pakistan, tomato is grown on area of 62.6 thousand hectares which produces 587.1 thousand tons with an average yield of 9.4 thousand tons per hectare [11]. In Jordan, according to the department of Statistics, Ministry of Agriculture [16], the average cultivated area was 19.63 Dunm with an average yield of 119.24 tonnes.

Earlier studies reported major important tomato diseases such as early blight (*Alternaria solani*) [5,13,17,18], late blight (*Phytophthora infestans*), and Septoria leaf spot (*Septoria lycopersici*). Some diseases have become the most important ones; these diseases include powdery mildew (*Leveillula taurica*), root knot nematode (*Meloidogyne spp.*) and bacterial wilt (*Ralstonia solanacearum*). However, Fusarium wilt of tomato is one of the most important diseases, which affect all plant stages (seedling stage, flowering stage, and fruiting stage). Also, it can affect the whole plant parts, leaves and stems [9,12,13,17,19-24]. Agrios [25] reported that the disease is most destructive in warm climates and warm sandy soil of temperate regions. The recorded diseases that attack different agricultural crops especially tomato in Jordan are shown in (Table 1).

Fusarium wilt around the world is considered as one of the most important diseases of tomato [17-19,21,22,24,26-29]. The causal agent of this disease is *Fusarium oxysporum lycopersici* [12,19,20,24,29-32].

It is a soil-borne phytopathogen causing vascular wilt by infecting plants through the roots, growing internally through the cortex to the stele, and killing the plants [20,23,24,29,32-

34]. Worldwide, tomato yield is reduced to 30 to 40% due to *F. oxysporum* [35]. To avoid these losses, several studied strategies have been used such as: (1) Disinfection of soil and planting material with fungicidal chemicals like benomyl, captafol, imazalil, thiram, etc. [36], (2) Crop rotation, (3) Use of resistant cultivars [37,38] and (4) Use of healthy biological control agents derived from microbes or medicinal herbs [3,20,23,24,29,30,32,33,39,40].

This review paper is aimed at summarization of tomato Fusarium wilt; a disease caused by *Fusarium oxysporum Lycopersici*. Different ways of management and control in general and in specific, the use of various medicinal plants extracts (ecofriendly) biological control methods in Jordan.

Tomato Plant: Botanical Notes

Tomato plant (*Lycopersicon esculentum* L.) which belongs to family *Solanaceae* and the genus *Solanum* is an annual plant, which can reach a height of over two meters [11,17,19,21,22,26-29,34]. The first harvest is possible 45-55 days after flowering, or 90-120 days after sowing. The color ranges from yellow to red [11,41]. Three different types of tomato plants can be distinguished as tall or indeterminate type, semi-bush and bush (determinate) type. The tall varieties are the best choice for a long harvest period, they exhibit a good feature called indeterminate; and that they keep growing after flowering. The more foliage the plant exhibits keeps the temperature lower within the crop and the fruits grow in the shade of the leaves. Hence, the sun does not damage the fruits and they ripen more slowly. Slower ripening and a high leaf/fruit ratio improve the taste of the fruits and its sweetness [41].

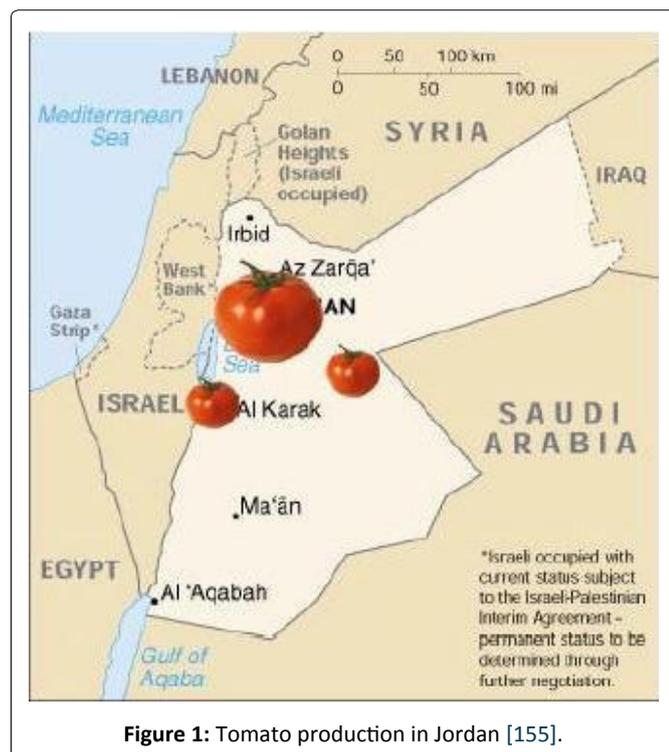


Figure 1: Tomato production in Jordan [155].

Tomato in Jordan: Cultivation and Economic Importance

Jordan is a regional tomato producer; and the world's 4th largest exporter of fresh tomatoes [42]. Important varieties grown in Jordan include Shams, RS589956, Jawaher and Casandra. Tomato is grown throughout the country, in outdoor fields, greenhouses and net-houses where irrigated water and arable lands is available. Tomatoes can be cultivated in different soil types and artificial well-draining substrates. Optimum day temperature for growth is 18 to 25 °C and night temperatures between 10 and 20 °C. Furrow and drip techniques are used for irrigation. Harvesting crop is done 70-140 days from transplanting depending on the crop variety and on cultivation conditions used. Tomatoes are planted throughout the year in Jordan as the following: Southern part of Jordan Valley: January-February; Middle part of Jordan Valley: May-June; Northern part of Jordan Valley: June-July and Safa Crop (hilly areas): July-November (Figure 1) [42].

In Jordan tomatoes production covers about 27.5% of the total vegetable cultivated area and about 3% of the total cultivated area. The highest vegetable production is for Tomato at 43.4% of total vegetable production in 2012 and is followed by cucumber (9.2%), potato (8.3%), eggplant (7.1%), and water melon (6.4%). According to the Department of Statistics, Agricultural Survey data, the total area under production of both greenhouse and open field, tomatoes in Jordan increased from 7,656 ha in 2002 to 12,345 ha in 2012, indicating a growth of 161%.

The total tomato cultivated area has increased from 11,752 ha in 2008 to 12,345 in 2012, reflecting a growth of approximately 5%. The average yield has increased from 51.7 ton/ha in 2008 to a good yield rate of 59.8 ton/ha in 2012 [43]. According to Department of Statistics, Ministry of Ag-

riculture in Jordan (2018) the average cultivated area was 19.628 ha with an average yield production of 119.242 tons reflecting a good yield increase of Tomato.

Importance and Use of Tomato Plant: Nutritive Value

Tomato is considered as highly nutritive, protective and the freshest vegetable with a widespread production in Jordan and in different countries around the world, [11,29,44]. It has much nutritional value at very cheap price as compared to other vegetables. It has 95.3% water, 0.07% calcium and niacin, Vitamin A, C, E and rich source of nutrients like Na, K, Fe, and antioxidants especially lycopene and salicylate. All of have great importance in human metabolic system. Every 100-gram of raw tomatoes contain 93.59% water, 22 calories, 1.1 g protein, 4.7 g carbohydrates, 13 mg calcium, 27 mg phosphorous, 0.5 mg ferrous, 244 mg potassium, 900 mg vitamin A 0.06 mg thiamine, 0.04 mg riboflavin, 0.7 mg niacin, 23 mg ascorbic acid [29,45]. In addition, the red pigment of the lycopene which tomato fruit contains has attractive interest because the lycopene has high antioxidant ability against oxygen radicals that may cause cancer, aging, arteriosclerosis, etc [11,41,46-50].

Fusarium Genus: Morphological Characteristics and Distribution

Fusarium is a filamentous fungus (*Sordariomycetes:Hypocreales:Nectriaceae*) containing different phytopathogenic and toxigenic species. The genus is highly diverse with twenty monophyletic species complex and outgroups of nine species. The commonest species include *Fusarium solani*, *F. oxysporum*, *F. equiseti* and *F. chlamydosporum* [18,51-53]. Key features for the differentiation of *Fusarium* species are color of the colony, length and shape of the macro conidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are [53].

Fusarium is one of the most important pathogenic fungi. Toxic metabolites such as enniatins and fusaric acid can be produced by different species of this genus causes severe diseases, and can contaminate agricultural product, making them unsuitable for food and feed. Moreover, trichothecenes can act as virulence factor in plant disease [53-55].

Diverse molecular methods can be used for rapid identification of *Fusarium* strains to species and sub-species levels such as 28S rRNA gene sequencing [56], polymerase chain reaction (PCR) based rDNA detection method [57] and detection of protein banding patterns by SDS-PAGE and esterase isozyme electrophoresis [18,53].

Fusarium Genus: Growth Characteristics

Windles [58] reported that the fungus grows on artificial medium on PDA. It grows rapidly covering 9 cm diameter Petri-dish in about 7 days and forms a hyaline, branching mycelium that is white to gray. On PDA medium isolates from diseased fruits formed light pink aerial mycelium and red pigment in the agar. On Czapek agar isolates formed colonies and mycelium appeared as aerial, grey to light purple in color

depending on the isolate. *Fusarium* cultures grown on Sabouraud Dextrose Agar medium at optimum temperature 25 °C produce cottony, flat, woolly, or spreading colonies [59,60].

Fusarium has diverse life cycles, niche specialization, host adaptation and specificity. *Fusarium graminearum* and *F. ver-*

ticilloides are pathogen that infects cereals, whereas *F. oxysporum* infects both monocotyledonous and dicotyledonous plants [61]. *Fusarium oxysporum* is asexual; others are both asexual and sexual with either self-fertility (homothallism) or out-crossing (heterothallism). *Fusarium* species produce meiotic (sexual) spores and at least three types of mitotic (asexual) spores. However, all *Fusarium* species do not produce all type of spores: also, less than 20% of *Fusarium* species reproduce sexually [11,53,62].

Fusarium oxysporum

Fusarium oxysporum lycopersici is one of destructive soil borne fungal pathogen, causing wilt disease in tomato [11,18,62]. It produces white aerial mycelium with pink, orange, red, blue, and purple pigmentation developing with age. *Fusarium oxysporum lycopersici* can produce three types of asexual spores, macro- and micro-conidia and chlamydospores depending on culture conditions (Figure 2). Macro-conidia are produced long, sickle-shaped, thin-walled, spores with several septa. Micro-conidia are abundant and are smaller single-celled, oval-shaped spores. The production of micro-conidia on short monophialides is a distinguishing characteristic. Chlamydo spores are thick-walled, round; dormant spores are formed singly or in pairs. It is typically produced in 2-4 weeks on old culture [11,13,62,63].

The life cycle of *F. oxysporum* (Figure 3) begins with a spore landing on a leaf surface of a host plant. When environmental conditions of temperature and available moisture are favorable, the spore germinates; its hyphae elongate and

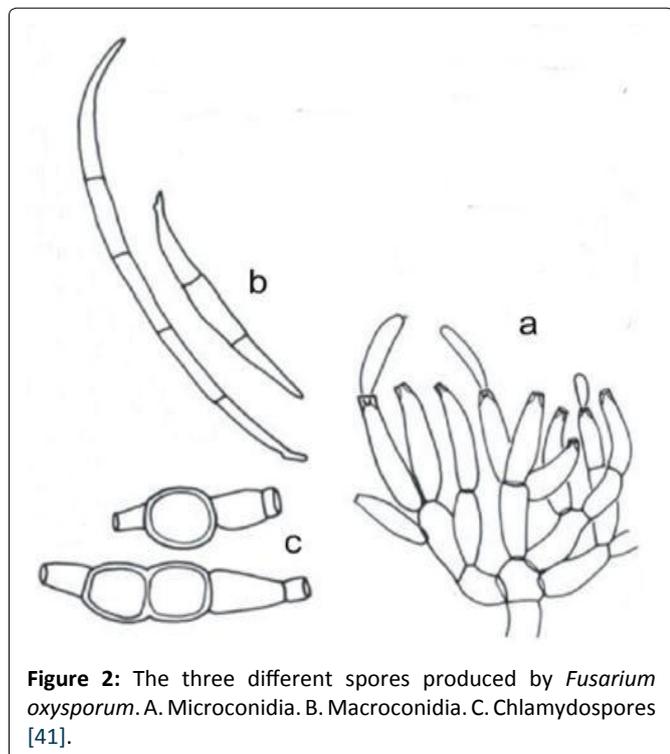


Figure 2: The three different spores produced by *Fusarium oxysporum*. A. Microconidia. B. Macroconidia. C. Chlamydospores [41].

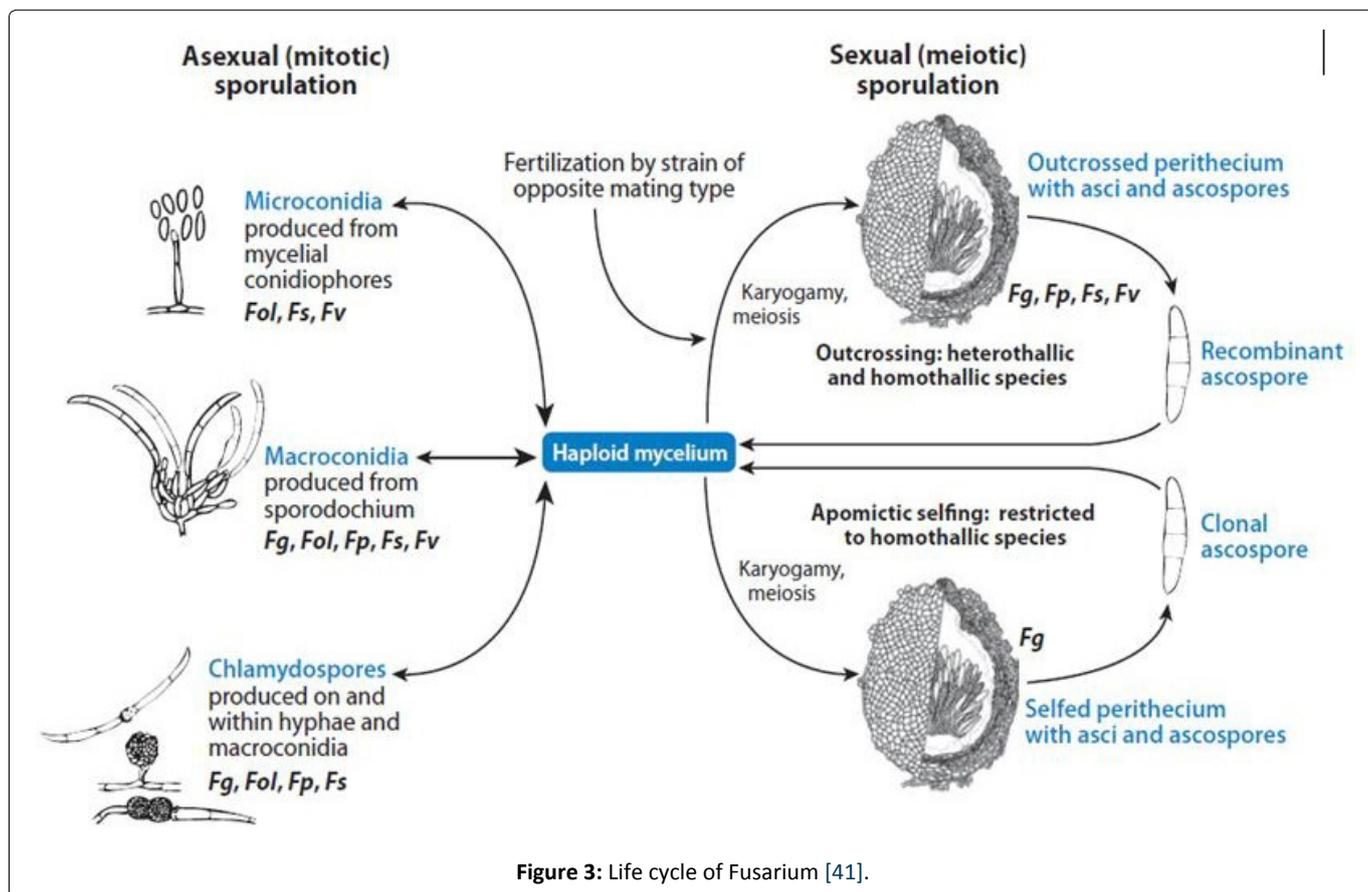


Figure 3: Life cycle of *Fusarium* [41].

penetrate into the leaf cuticle, stomata, or wounds. Once hyphae enter the leaf, the infection process begins. During the latent stage, the affected leaf area does not show any symptom until a length of time known as the incubation period has elapsed. The affected leaf area then develops symptoms and the pathogen begins to sporulate during the infectious stage [4,62,64].

F. oxysporum is the causal agent of vascular wilt, a disease that affects a large variety of important crops worldwide [12,18-20,29-32,62,64,65]. The high level of host specificity of pathogenic strains in *F. oxysporum* led to the development of the “*formae speciales*” concept to enable better differentiation of these morphologically similar strains [1]. The “*formae speciales*” are characterized by their ability to cause a wilt disease on a limited taxonomic range of host plants [18,62,64,66].

Strains of *F. oxysporum* which were isolated from healthy parts are called non-pathogenic and can protect plants against the pathogenic ones. Several non-pathogenic strains of *F. oxysporum*, isolated from soils suppressive to *Fusarium* wilt, have been selected as potential biological control agents [53,67]. In Jordan; there are three known physiological races of *F. oxysporum lycopersici* that are distinguished by their differential pathogenicity to tomato cultivars containing race-specific dominant resistance genes [68]. According to Al-Khatib [69], only races 1 and 2 were recovered. A total of 33 varieties were imported from various sources to investigate the effect of this disease and determine the source of their resistance during the period 1998-2002.

GS12 F1 Variety was the highest amount of seed imported with an imported amount of 275 kg, followed by Wafa F1 variety (70 kg), Guardian F1 (65 kg), Super Red F1 (37.5 kg) and RS589956 (33 kg) (National Center for Agricultural Research and Technology Transfer, Seed Certification Unit, 2003) in Alkhatib, et al. [69].

Fusarium wilt disease

Fusarium wilts disease caused by *F. oxysporum lycopersici* is one of the most prevalent and damaging diseases of to-

mato plant that causes considerable losses, under favorable weather conditions [18,20,23,24,29,32,34,62,64,69,70].

Several studies tried to explain wilting of tomato plant infected with *Fusarium oxysporum lycopersici*. Gaumann [71] suggested that wilting results from destruction of permeability of leaf cell by fungal toxin. Other reports suggested that wilting is caused by occlusion of xylem vessels by mycelia and conidia, tyloses, gums and hydrophilic material that may form gels in vessels [64,72]. However, Gothoskar, et al. [73] reported that wilting tomato plant is due to increased viscosity of tracheal fluid and eventual mechanical plugging of vessels by pectic gels. Chambers and Corden [74] found that in diseased stems and petioles, the vascular bundles fail to increase in size and few vessel elements that are produced remain small and collapsed. Horsfall and Dimond [75] reported that the blocking of the xylem ducts, the toxins or enzymes carried along with the flow sap are responsible for wilting.

The pathogenic fungus moves in infected plants through the root system and grows internally through the cortex to the stele using its sporangial germ tube or mycelium [20,76]. The root can be infected through root tips, wounds in roots or at the formation point of lateral roots. Mycelium enters the xylem vessels branches and forms microconidia spores [20]. Fungal growth in the vascular system affects water supply which induce the leave stomata to close and plugging the vascular system which results in leave wilting and the plant dies (Figure 4) [18,20,53,64,77].

Disease Development

Disease development is favoured by warm temperatures (27-28 °C), dry weather, and acidic soil (pH 5-5.6). Chlamydo spores stay dormant and immobile in the remains of decayed plant tissue until stimulated to germinate by utilizing nutrients that are released from extending roots of a variety of plants [18,62,64,77-80].

Invasion of the roots is followed by the penetration of the epidermal cells of a host or a non-host and the development of vascular disease in host plants [81]. The process of vascular infection by *F. oxysporum* includes adhesion in which fungal



Figure 4: Fusariums wilt of Tomato caused by *Fusarium oxysporum lycopersici* (a) Infested farm land (b) Vascular discoloration stem [53].

infection starts when hyphae adhere to the host root surface [77,82]. Penetration is controlled by a combination of different factors that include fungal compounds, plant surface structures, activators or inhibitors of fungal spore germination, and germ tube formation. Some pathogenic forms penetrate roots directly, whereas others must enter indirectly through wounds [65,83].

The last stage is colonization; the mycelium advances intercellular through the root cortex until it reaches the xylem vessels and enters them through the pits. Fungal colonization of the host's vascular system is facilitated by the formation of microconidia within the xylem vessel elements that are detached and carried upward in the sap stream [4,18,20,62,64,84].

Virulence genes in *Fusarium oxysporum*

Indurum and Howlett [85] reported about 79 genes described at that time, and divided them into several categories, depending on their involvement in the formation of infection structures, cell wall degradation, response to the host environment, toxin biosynthesis, signal cascades, and novel functions. However, studies on genes related to pathogenicity in *F. oxysporum* have been limited. Two of the most important virulence genes associated with *F. oxysporum* is FOW1 and ARG1.

Manikandan, et al. [86] attempted to realize the mechanism of wilt caused by *F. oxysporum*, the total proteome of twenty isolates were analyzed along with the cultural, morphological, virulence and molecular characteristics. The seventeen different proteins showed by 2D analyses they reported the occurrence of the FAD binding domain containing protein, Cutinase-2, Chaperone, Cytochrome P450, sulfate anion transporter, Glycoside hydrolase family 85 protein, 60S ribosomal protein and, ATP-dependent RNA helicase. These are the key proteins in virulence, symptom and wilt develop-

ment. These proteins were involved in sporulation, growth, maintenance of genome integrity and maximum penetration rate on host root tissues.

The occurrence of *Fusarium* wilt diseases is affected by soil temperature, since there is a strong relationship between substrate temperature and disease intensity; indicating that there were low and high temperature extremes at which wilt symptoms did not develop, and an optimum temperature at which the most severe disease occurred. The optimal growth of *F. oxysporum* was found to be between 25 °C and 28 °C [34,64,77].

Moreover, germination of chlamydospores of *F.oxysporum* is influenced by soil pH. Mycelia of *F. oxysporum* grew within the range of pH 2-12 [77]. Wilson [87] reported that acid soil (pH 4.2) supported growth of *Fusarium* through the soil, whereas a pH near neutrality prevented this growth. Woltz and Jones [88] concluded that *Fusarium* wilt disease is associated with acidic, sandy soils, rather than heavier soils with higher pH values. *Fusarium* growth stages in soil depend on the ecological balance and nutrient availability. *F. oxysporum* is an autotroph, requiring only a carbon source for structure and energy, and inorganic compounds to synthesize organic compounds such as sugars, lipids and amino acids [88].

Growth, sporulation and virulence of *F. oxysporum* need essential nutrient elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, magnesium, sulphur, iron, manganese, molybdenum, and zinc [89]. High levels of nitrogen fertilization in agricultural soils lead to an increase in *Fusarium* wilt development [90].

Symptoms of *Fusarium oxysporum* and disease development

Fusarium wilt caused by the soil borne fungus *Fusarium oxysporum lycopersici*, was the most prevalent and damag-



Figure 5: Symptoms of *Fusarium* wilt on tomato plant in open field [48].

ing disease of tomatoes [20,23,24,29,32,34,77,91] (Figure 4 and Figure 5). *F. oxysporum* enter through the roots and interfere with the water conducting vessels of the plant. As the infection spreads up into the stems and leaves it restricts water flow, causing the foliage to wilt and turn yellow. Symptoms appear later in the growing season and are first noticed on the lower (older) leaves. As the disease progresses, the younger leaves will also be affected (leaves start drooping, curve downwards and turn brown yellow) and the plant eventually dies (Figure 6) [4,34,64,77,92].

Wellman [93] described early symptom of tomato wilt and it is believed to be the first above-ground indication of infection of the tomato by wilt pathogen. Foster [94] reported that the early symptom is clearing of the ultimate veinlets in the leaflets of infected tomatoes giving them a netted appearance. Horsfall and Dimond [75] reported that tomato plants affected by *F. oxysporum lycopersici* show severe break down on vascular tissue only in the last stages of disease and then only if the plant is young (Figure 6b).

Management of *Fusarium* wilt

The control of *Fusarium* wilt of tomato is important in maintaining plant vigour and fruit quality and quantity [62,77]. Integrated disease management (IDM) is an approach used to maintain plant health by minimizing damage caused by diseases [95]. Control principles and methods of *Fusarium* wilts should be targeted to excluding the pathogen, as well as reducing the amount and/or efficiency of the initial inoculum. Therefore, IDM strategies include: (1) Use of pathogen-free planting material, (2) Site selection to avoid planting into high risk soils, (3) Reduction or elimination of *F. oxysporum* inoculum in soil, (4) Use of bio-control agents for protection of healthy planting material from infection by res-

ident or incoming inoculum subsequent to planting, (5) Use of resistant cultivars regardless the level of resistance, and (6) Choice of cropping practices to avoid conditions favoring infection of the plant [96].

Pathogenic strains are characterized by their ability to invade and colonize the vascular system of the host plant. Integrated disease control methods can be applied by the application of biological, chemicals, cultural, physical, and regulatory methods, depending of the nature of the agents used [62].

Cultural practices were reported by different authors, Agrios [27] reported that seedbed sterilization and crop rotation has reduced infection by *Fusarium*. Jones and Overman [97] noticed that rotations of up to 5-7 years can significantly reduce soil inoculum levels. The type of soil has also affected reduction on inoculum potential of fungus. Jones and Overman [97], Walker [98] and Jones, et al. [99] showed that soil pH and fertility can also have significant effect on the severity of *Fusarium* wilt. Agrios [25] reported that the use of healthy seed and transplants is mandatory and hot-water treatment for seed should precede planting.

Chemical Methods

Soil treatment with broad-spectrum fumigants such as methyl bromide, chloropicrin, or methyl isothiocyanate successfully controlled *Fusarium* wilt of tomato and increased crop yield [11,12,22,32,33,62,100,101]. Anon [102] and Ristaino and Thomas [103] reported that methyl bromide fumigation is used for tomato production in some geographical areas. Biehne [104] found that benomyl applied to tomato plants 2 to 3 days after inoculation with *Fusarium* has reduced wilt symptoms approximately 70% in 2.5 to 3 weeks after inoculation.

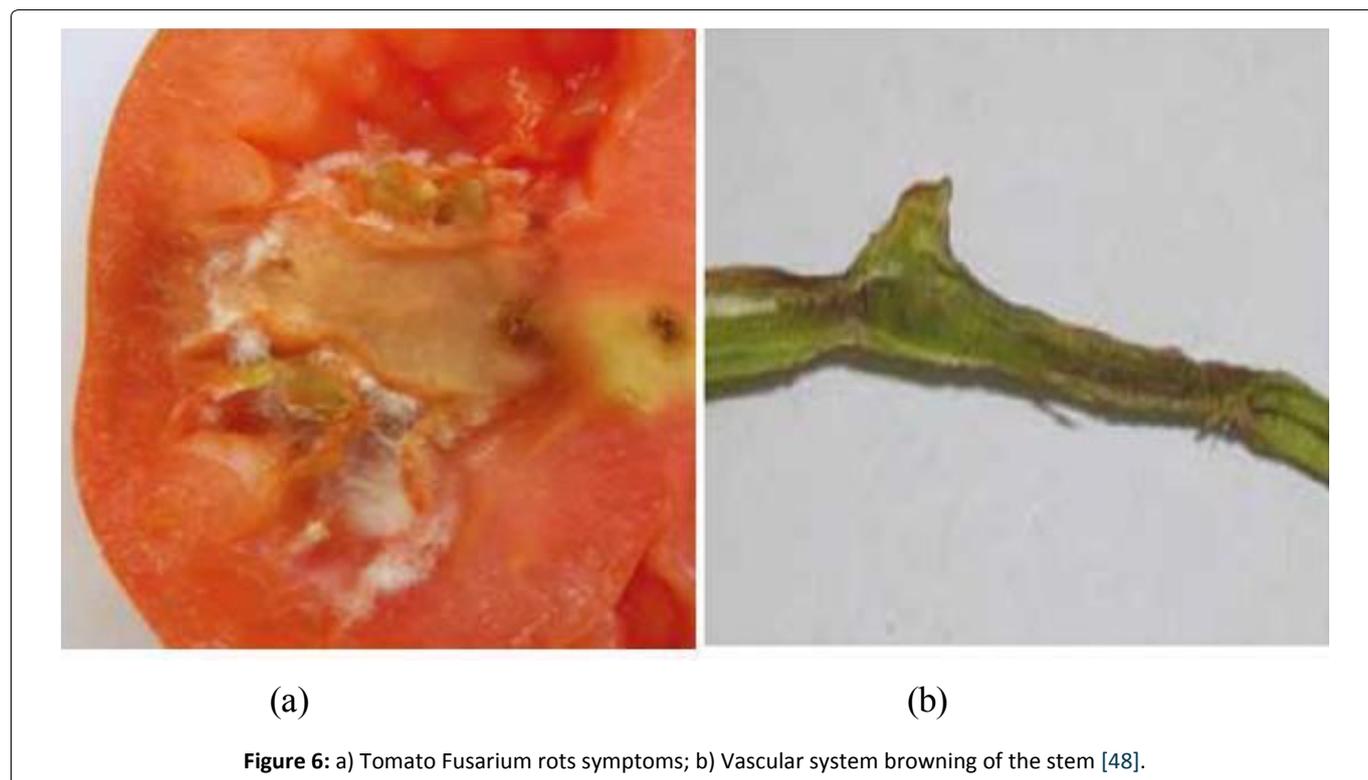


Figure 6: a) Tomato *Fusarium* rots symptoms; b) Vascular system browning of the stem [48].

Limiting the uptake of benomyl to 6 to 11 days before inoculation, however, resulted in only 7% reduction in disease symptoms. However, the efficiency of soil fumigation is affected by either survival of pathogens in soil layers below the depth of effective fumigation, or reintroduction of them through infected planting material or by conidia carried in the air or irrigation water [105]. Also, methyl isothiocyanate is used to enhance biodegradation in soil by adaptation of microbial populations to use the compound as an energy source [106].

Other chemical fungicides such as prochloraz and carbendazim [107], bavistin [108] and salicylic acid [109] are used to suppress the disease by inducing resistance, but these chemicals have a negative impact on human health and are hazardous to the environment. Pathogen resistance could emerge against these fungicides under certain circumstances. Moreover, the residual of many fungicides could damage the ecosystem and negatively affect human life if they reach our diet or water [17,44,53,110].

Physical Methods

Soil solarization, flooding and sanitation are physical ways used for the control of *Fusarium* wilt diseases [32,62]. Soil solarization is a hydrothermal process that occurs when moist soil is covered with thin (25 to 50 μm), transparent plastic polyethylene or polyvinyl sheets during a period of high temperature and intense solar radiation [12,22,96]. It was used to reduce the population density of *Fusarium* species in soil by 88 to 93, and provided effective control *Fusarium* wilt diseases on tomato [20]. Katan, et al. [111] noted that soil solarization for 2 weeks during summer time in Israel reduced the populations of buried inoculum of *F. oxysporum vasinfectum* and *Verticillium dahliae* by 94 to 100% at 5 cm, 67 to 100% at 15 cm, and 54 to 74% at 25 cm. Effective disease control was obtained with maximum temperatures within the range of 45 to 50 °C and 38 to 45 °C at depths of 10 and 20 cm respectively [96]. Flooding is a soil disinfection method which harms soil-borne pathogens by reduction of O₂, increase of CO₂, or a diversity of microbial interactions that result in toxic substances to pathogens upon anaerobic processes [95].

Sanitation includes practices that remove and destroy sources of inoculum from affected plants or infested debris. Thus, flaming residues of affected crops to achieve thermal killing of pathogen resting structures would reduce that effect and reduce disease risk in host crops. Flaming crop debris with propane or oil fuelled flammers allow more controlled heating similar to thermo-sanitation with lesser environmental effect [32,96].

Genetic Methods: Use of Resistant Cultivars

The use of resistant cultivars is one of the most practical, cost-efficient, and environmentally safe control method for the management of *Fusarium* wilt diseases. However, several factors can limit its use and effectiveness, including genetic and pathogenic variability, and the evolutionary pattern of the pathogen, availability of resistance sources, and co-infection of the plant by other pathogens, genetics and penetration of resistance [11,22,62,77,96]. The use of resistant

varieties is an important method, which is reliable and cheap for management of plant disease but due to development of new races of the pathogen, the resistant variety becomes susceptible one [12,17].

According to Pritesh, et al. [112], identification and utilization of tomato plant varieties resistant to the disease represents an alternative choice to the use of chemicals. The advantages of this method include saving the cost of chemical for control of the disease and enhancing cultivation of previously infested field. But, due to breakdown of resistance in the face of high pathogenic variability in the pathogen population, the usefulness of many resistant cultivars is restricted to only a few years [113,114].

In Jordan, Al-Khatib, et al. [69] studied the local tomato cultivars resistance to *Fusarium oxysporum lycopersici*, Twenty-four cultivars were inoculated with composite samples of fungal suspensions from race 1, race 2, and equal volumes of race 1 and race 2 isolates of *F. oxysporum lycopersici* using the standard root dip method. The effect of tomato growth stage at the time of infection with the vascular wilt fungus *F. oxysporum lycopersici* was investigated. The age of tomato plant played an important role in its ability to overcome the disease, and there were significant differences among plants inoculated soon after transplanting, one, two, and three weeks after transplanting, compared to plants inoculated four weeks after transplanting which did not show any significant differences from the non-inoculated control under experimental conditions.

Biological Control

In an attempt to reduce the use of chemical pesticides, there is an increasing interest in introducing several biological control agents and plant compounds as natural commercial products for managing soilborne pathogens. It is generally ideal, safer and has a minimal environmental impact [11,17,22,32,62,76].

Fuchs, et al. [115] reported that several biological control agents including bacteria and non-pathogenic strains of *Fusarium oxysporum* have shown promise for the control of *Fusarium* wilt of tomato. Different *Trichoderma* species are effective biological control agents against a range of crop diseases especially *Fusarium* wilt pathogen of tomato [32,116,117]. The capability of *Trichoderma* spp. to suppress plant diseases is due to their direct antagonistic effects on the fungal pathogen, and especially their ability to produce lytic enzymes e.g. chitinases and β -1, 3-glucanases [118,119]. These enzymes hydrolyze the pathogen's cell wall thereby limiting the growth of fungal pathogens [118].

Panteleev [120] reported that coating seed with culture of *T. viridae* lowered incidence of *F. oxysporum lycopersici* on tomato from 29.5 to 6-15%. Gail, et al. [121] and Jayalakshmi, et al. [122] reported that the activity of both polyphenoloxidase and peroxidase increased after the treatment with *T. harzianum*, and also with El-Khallal [123]; Mandal, et al. [124], since they showed that the activity of POD was increased in response to foliar spray of salicylic acid. Spraying of salicylic acid increased the activity of the enzyme in *Trichoderma* seedling

root dipping treatment not in soil applied treatment. Combination of salicylic acid and *Trichoderma* as seedling root dipping and thiophanate methyl with its half recommended rate recorded a higher PPO and POD activity.

Different bacteria were reported as they capable to control various plant diseases [32,118,125]. Pathogen suppression methods by the antagonistic bacteria include direct parasitism, antibiotic production, and substrate competition and induced systemic resistance in the plant host [126,127]. The cell wall-degrading enzymes, as chitinases, glucanases, and proteases are the major lytic enzymes that are secreted by different biocontrol agents [128]. These enzymes attack the cell walls of phytopathogenic fungi, causing cell lysis and ends with death [129,130]. Moreover, the production of volatile antimicrobial compounds by the antagonistic bacteria and fungi has been well reported [131].

Cruz-Rodríguez, et al. [55] studied The *C. longirostrata* branch extract efficacy for the control of *Fusarium* wilt in maize. The application of the extract reduced the percentage of disease incidence significantly caused by *Fusarium verticillioides* from 70.4% to 40.12% as compared to non-treated plants, and the disease severity was reduced from 40.15% to 29.46%.

Plant extracts and plant essential oils have been reported to be effective antimicrobials against food and grain storage fungi, foliar pathogens and soilborne pathogens [23,34,76,132]. These are good alternatives to chemical pesticides, as they are readily biodegradable in nature [4,23,62].

Garlic (*Allium sativum*) is a popular spice, is also known for its medicinal uses as an antibiotic, anti-thrombotic and antineoplastic agent. Garlic contains at least thirty-three sulfur compounds, several enzymes and seventeen amino acids. Additional ingredients of intact garlic include steroidal glycosides and lectins [132].

Activity of garlic extract toward *Fusarium* wilt is due to sulfur-containing compounds such as ajoene or allicin. Sprays with aqueous garlic extract have antibiotic and antifungal properties and will suppress a number of plant diseases, including powdery mildew on cucumbers and, to some extent, black spot on roses. Garlic extract controlled diseases such as mildew, rusts, fruit rots, blights, and black spot [62,132]. Garlic releases fungicidal chemicals into the soil. Its extract shows high inhibitory activity against *Aspergillus niger*, *Penicillium cyclopium* and *F. oxysporum* for all tested concentrations. Allicin is the active antimicrobial component of garlic extract; it is a natural product used to treat several plant pathogens [54]. Allicin is known as 2-propene-1-sulfinothioic acid S-2-propenyl ester; thio-2-propene-1-sulfinic acid S-allyl ester. Obagwu and Korsten [133] noticed that using garlic extract was an effective method to control green and blue molds of citrus caused by *Penicillium digitatum* and *P. italicum* and they found that garlic extract can inhibit growth and development of mycelia for both pathogens significantly.

Coventry, et al. [134] reported that neem (*Azadirachta indica*) extract showed antimicrobial activity with recorded effects on some fungal pathogens. Kimaru, et al. [135] revealed

that neem cake powder contains ingredients that have fungistatic effects against *Fusarium* wilt of tomatoes. Agbenin and Marley [136] reported that crude extracts of neem and garlic at concentrations ranging from 5% to 30% of the material in 100 ml of potato Dextrose Agar inhibited mycelial growth of *F. oxysporum lycopersici* at various levels. Dry neem seed extract gave 100% inhibition. In a study of Agbenin and Marley [136], they reported the effect of different extracts of neem leaf, neem seed and garlic at concentrations ranging from 5% to 30% of the material in 100 ml of Potato Dextrose Agar on mycelial growth of *F. oxysporum lycopersici*.

All the extracts inhibited mycelial growth at various levels. Dry neem seed extract inhibited mycelial growth 100%. Fresh neem leaf extract reduced mycelial growth with increasing concentration while in garlic there were no differences in growth inhibition among the various concentrations used. However garlic extracts decreased sporulation with increasing concentration and cultures grown on extract amended agar plates.

Hanaa, et al. [137] investigated the effect of neem and willow (*Salix babylonica*) 10% aqueous extracts on *Fusarium* wilt disease in tomato and revealed that the percentage of disease incidence was reduced to the level of 25.5% and 27.8% after 6 weeks of infection. Also, fresh neem leaf extract reduced mycelial growth with increasing concentration while in garlic; there was no difference in growth inhibition among the various concentrations used.

In the study of Jun Ma, et al. [138], garlic and black pepper extracts each at 0.5% and 4% concentrations were used as natural resistance inducer treatments for treating 4-week-old tomato seedlings. The highest concentration (4%) of garlic and black pepper was significantly better than the lowest concentration (0.5%) for reducing the percentage of wilted plants and wilt disease severity comparing to the untreated inoculated control.

Mishra, et al. [20] noted that the extract of goat weed (*Ageratum conyzoides*) exhibited maximum toxicity (96%) against *F. oxysporum lycopersici*. Significant results were also observed with extracts of floss flower (*Ageratum houstonianum*), glory bower (*Volkameria inermis*) and beleric (*Terminalia bellirica*) showing inhibition of 90%, 85% and 79%, respectively.

Yeole, et al. [23] screened seven plant extracts against *F. oxysporum*; cinnamon (*Cinnamomum zeylanicum*); hing (*Ferula foetida*); anantmul (*Hemidesmus indicus*); pushkarmool (*Inula racemosa*); manjishtha (*Rubia cordifolia*); kushta (*Saussurea lappa*) and clove (*Syzygium aromaticum*). Methanol (MeOH) extract of clove showed largest zone of inhibition; solvent extracts of cinnamon and clove exhibited 100% inhibition of *F. oxysporum* spores at 5 and 10 mL.L⁻¹ concentration. The active compounds (metabolites) in the methanolic clove extracts were separated and identified as eugenol and 1-heptatriacotanol.

Earlier studies reported that eugenol inactivate several enzymes, cell membrane integrity and affect genetic material [139]. Also, being a lipophilic compound, eugenol can enter

the fatty acid chains in the membrane lipid bilayer and alter fluidity and permeability of cell membranes [140]. Akaze, et al. [49] reported that methanol extracts of *A. indica* at low concentration of 12.5% showed more bioactivity against *F. oxysporum lycopersici*.

Ohunakin and Bolanle [141] evaluated aqueous extracts of garlic, ginger (*Zingiber officinale*) and wormseed (*Dysphania ambrosioides*) against *F. oxysporum. A. sativum* at 100% gave the highest inhibitory effect (71.24%) on mycelial growth, and compete with carbendazim (80.38%). Hot water extraction revealed better antifungal effects (45.86%) on *F. oxysporium* than cold water extract (33.80%). *A. sativum* showed the highest inhibition of 71.24% and 66.92% at 80% and 100% respectively, after carbendazim (80.38%) at 0.5 mg/ml which is the standard. However, all extracts recorded the lowest mycelia growth at 20% concentration level.

Sreenu and Zacharia [142] investigated the antifungal activity of aqueous extract of neem leaf (5%), ginger bulb (5%), West Indian lantana (*Lantana camara*) (5%), *Trichoderma harzianum* (5%), carbendazim (0.01%) and combination of carbendazim + *T. harzianum* (0.01 + 5%) against *F. oxysporum lycopersici* *in vitro*. The least growth of pathogen was recorded in carbendazim (treated control) followed by neem leaf followed by Lantana camara ginger bulb and there was no growth in carbendazim treatment in poison food technique.

Nasrin, et al. [34] studied biological and chemical control of *Fusarium oxysporum lycopersici* by nine plant extracts, three fungicides (Sulcox, Indofil M 45 and Ridomil MZ 68) and three antagonistic fungi. They reported that all plant extracts at 25% concentration were effective in reducing the mycelium growth of *Fusarium oxysporum lycopersici*. Antagonistic effect of *Trichoderma* sp. shows the highest percent inhibition radial growth (82%) as compared to *Sclerotium* sp. and *Aspergillus* sp. against *Fusarium oxysporum lycopersici*. Chemical treatment with Sulcox was proved that the most effective fungicides against this pathogen.

Plant essential oils are concentrated volatile hydrophobic liquids extracted from different parts of the characteristics according to the identification keys of aromatic plants [143]. Clove oil has been known as organic pesticide derived from different parts of the clove plant [144]. It has useful antimicrobial effects on different plant pathogens as fungi, bacteria and nematodes [62,145]. Thabet and Khalifa [145] investigated the inhibitory effects of different concentrations of clove oil on the mycelial growth and spore germination of all isolated fungi. Clove oil exhibited an inhibitory effect against the mycelial growth of all pathogens. All tested concentrations reduced mycelial linear growth of the tested fungi compared to respective controls significantly. Complete growth inhibition was observed in *F. oxysporum* and *Rhizoctonia solani* when clove oil was applied at concentration of 4%.

The phyto-constituents obtained from piper species are characterized by the production of typical classes of compounds such as amides, benzoic acids, chromenes, terpenes, phenylpropanoids, lignans, other phenolics and a series of alkaloids. They have shown anti-feeding, antibacterial, antifungal, antiplatelet, antioxidant, anti-inflammatory, antiame-

bic, insecticidal, cytotoxic, antiplasmodial and DNA damaging activities [146]. *P. nigrum*, known as black-pepper, has shown great potential for the discovery of novel biologically active compounds and need for techniques to enhance the production of high quality consistent plant material for accumulation of metabolites [147].

Effectiveness of crude chloroform extract of betel (*Piper betle*) in controlling *Fusarium* wilt of tomato was observed by Singha, et al. [12]. It was observed that 1% (w/w) amendment of the crude chloroform extract of *Piper betle* L. (PbC) in soil was more efficient in reducing the *Fusarium* population in soil than carbendazim and the combined amendment of carbendazim and chloroform extract of *Piper betle* L (PbC). Higher accumulation of total phenolics was observed in the *Fusarium*-infested plants as compared to that of healthy control and PbC-treated plants. Moreover, it was observed that the extract could reduce the symptoms and disease development.

In Jordan, Sidawi, et al. [148] evaluated the efficiency of methanolic extracts of the following plants: fig (*Ficus carica*) leaves, myrtle (*Myrtus communis*) leaves, and stem, leaves, flowers and roots of marigold plant (*Tagetes patula*) separately to control *Fusarium* wilt and root rot disease on tomato plants. They concluded that both extracts of marigold stem and leaves with 6% concentration significantly reduced the percent of infected plants. In another study, Al-Hussain, et al. [44] recommended the use of garlic plant extracts to control *Pythium ultimum* on tomato seedlings since it is considered as an environmentally friendly product. Undiluted garlic extract showed the highest control activity with no growth as compared to the biotic control without the extract whereas diluted garlic extracts 10 and 5% reduced the fungal growth to 15.5 and 41%, respectively.

Several studies report that soil amendments with composts control several important soil-borne pathogens including *Fusarium* wilts [101]. These composts suppress plant diseases through biotic and abiotic factors that reduce disease severity [149]. Populations of non-pathogenic strains of *Fusarium oxysporum* and fluorescent *Pseudomonas* spp. were shown to be contributed in the suppressiveness process toward *Fusarium* wilt [101]. Abiotic properties, such as the nature of any clays and pH, interact with these microbial populations that support suppressiveness effect [67,149].

Ros, et al. [101] assayed the biopesticide effect of four green composts against *Fusarium* wilt in melon plants and the effect of soil quality in soils amended with composts. Green composts showed high advantageous characteristics; improved plant growth and controlled *Fusarium* wilt in melon plants.

Al-Hussain, et al. [150] concluded that combinations of bio-control agents and resistance inducer could provide promising integrated alternatives in suppression of *Fusarium* wilt disease of tomato plants due to number of mechanisms involved. Naing, et al. [151] concluded that bacterial strain *Paenibacillus ehimensis* KWN38 showed high antifungal activity against six tested fungal pathogens belonging to various taxonomic groups; the strain produced volatile antimicrobial

compounds which had strong fungal growth inhibitory effect. The strain also showed high chitinase, cellulase, glucanase and protease activities.

Kouki, et al. [152] investigated the efficiency of both compost of vegetable waste and *Posidonia oceanica* mixture (70:30% v:v) against *F. oxysporum radialis-lycopersici*, The incorporation of non-sterilized vegetable *posidonia oceanica* compost (VPC) in the culture medium showed potent anti-fungal activity against *F. oxysporum* and complete inhibition of mycelium growth was observed for all the tested compost rates (0.5, 1, 2, 4, 6, 8, 10, 15 and 20%). *Bacillus sphaericus* (B12 and BS2), *Pseudomonas putida* PPS7 and *Burkholderia gladioli* BuC16 bacterial strains protected significantly tomato against *F.oxysporum radialis-lycopersici* attacks.

Salem, et al. [149] also evaluated the compost effectiveness on *Zea mays* and *Hibiscus sabdarriffa* under *Fusarium* wilt disease. A significant decrease was observed for both *Z. mays* and *H. sabdarriffa* compared to infected plants without compost. The observed disease suppression in compost-amended soil was associated with the reduction in soil pathogen population and increase in microbial activity of composts. Moreover, diversification of different organic materials in compost enhanced the activation of the microbial population in soil that eventually increases disease suppression and effectively controlling *Fusarium* wilt.

In another study of Hasan and Abo-elyousr [153] suppressive effects of six compost types on *Fusarium* wilt disease were studied under greenhouse conditions. Soil treatments with Khaya and Eucalyptus composts significantly reduced the infection percentage and disease severity of basil wilt. Otherwise, the applications of *Araucaria*, *Datura*, *Ficus* and *Azadirachta* composts showed no effect on both infection percentage and disease severity.

Juber, et al. [31] evaluated the efficiency of biocontrol agents (*Bacillus mycooides* and *Trichoderma viride*) and the chemical compounds, Acibenzolar-S-methyl (ASM) and Preservepro against *F.oxysporum lycopersici* in culture media and under greenhouse conditions. The results showed that all the control agents exhibited an inhibition rate on *F. oxysporum* growth on both culture media and under natural conditions. The addition of bio- and chemical agents to the contaminated soil induced significant reduction in disease incidence and disease severity associated with increase plant dry weight.

Markakis, et al. [67] assayed the suppressive effect of six different compost amendments (A, B, C, D, E and Z) against *Fusarium oxysporum radialis-cucumerinum* (Forc) in cucumber and *Verticillium dahliae* in eggplant. It was shown that composts A, B, C and D were effective against Forc, and composts C, D and Z were effective against *V. dahliae*. The decreased symptom severity and *V. dahliae* isolation ratio in eggplant was associated with significantly lower accumulation of phenols in stem tissues; whereas the concentration of total phenols in stem tissues of *V. dahliae*-infested eggplants was significantly higher compared to the non-infested.

Cruz-Rodríguez, et al. [55] evaluated the *C. longirostrata* branch extract for the control of *Fusarium* wilt in maize. The

application of the extract reduced the percentage of disease incidence caused by *Fusarium verticillioides* from 70.4% to 40.12% as compared to non-treated plants, by which, disease severity was reduced from 40.15% to 29.46%. The phytochemical components of the extract were cinnamic acids (caelic acid and ferulic acid) and phenolic acid (gall acid).

Conclusion

Effective disease management is best achieved by quick diagnosis and practicing integrated disease management strategies. Use of pathogen free seed of wilt resistant cultivars is most successful, practical and cost efficient measure for disease management. Moreover, additional work for discovering of genetics and host-pathogen interaction are needed to produce high yielding wilt resistant cultivars. Combined use of wilt resistant cultivars delayed planting, seed dressing with fungicides, avoidance from dense planting, and use of bio-control agents help in reduction of disease incidence.

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